CLAIMS

1. A method, comprising:

synthesizing one or more nucleic acid sequences relevant for clinical reference;

tagging at least one end of each sequence for amplification by a primer; and

amplifying the one or more nucleic acid sequences using the primer.

2. The method as recited in claim 1, wherein the tagging includes attaching an additional sequence of nucleotides, wherein the additional sequence is complementary to a nucleotide sequence of the primer.

3. The method as recited in claim 1, wherein the tagging includes attaching an additional sequence of nucleotides, wherein the additional sequence is identical to a nucleotide sequence of the primer.

4. The method as recited in claim 1, wherein:

the tagging includes attaching a first sequence of nucleotides to a first end of each of the one or more synthesized nucleic acid sequences, wherein the first sequence is complementary to a nucleotide sequence of a first primer of a primer set, and

the tagging includes attaching a second sequence of nucleotides to a second end of each of the one or more synthesized nucleic acid sequences, wherein the second sequence is identical to a nucleotide sequence of a second primer of a primer set.

5. The method as recited in claim 1, wherein the synthesizing comprises synthesizing two complementary nucleic acid strands, wherein:

a first strand includes a first nucleic acid sequence relevant for clinical reference and a nucleic acid tag complementary to a first primer of a primer set and

a second strand includes a nucleic acid sequence complementary to the first strand and a nucleic acid tag complementary to a second primer of a primer set.

6. The method as recited in claim 5, wherein the first and second strands are synthesized as a duplex of complementary strands.

7. The method as recited in claim 1, wherein at least one of the one or more synthesized nucleic acid sequences includes at least one mutation of a nucleotide in a normal human nucleic acid.

8. The method as recited in claim 1, further comprising synthesizing multiple mixtures of at least one reference nucleic acid apiece, wherein:

each of the multiple mixtures has an associated primer set, and wherein:

each member of one of the multiple mixtures includes a first tag attached to a first end of the member, wherein:

the first tag comprises a sequence of nucleotides complementary to a nucleotide sequence of a first primer of the associated primer set, and

each member includes a second tag attached to a second end of the member, wherein:

the second tag comprises a sequence of nucleotides identical to a nucleotide sequence of a second primer of the associated primer set.

9. The method as recited in claim 8, further comprising combining the multiple mixtures and controlling each of the multiple mixtures to achieve separate amounts of amplification for each of the multiple mixtures.

10. The method as recited in claim 9, wherein controlling each of the multiple mixtures includes controlling an amount of each associated primer set for each of the multiple mixtures.

11. The method as recited in claim 9, wherein controlling each of the multiple mixtures includes controlling a physical characteristic of a combined mixture of the multiple mixtures to favor an amplification capability of one primer set over an amplification capability another primer set.

12. The method as recited in claim 9, wherein controlling each of the multiple mixtures includes controlling a physical characteristic of a PCR reaction to favor an amplification capability of one primer set over an amplification capability another primer set.

13. The method as recited in claim 9, wherein one of the multiple mixtures includes only one reference nucleic acid.

14. The method as recited in claim 1, wherein the synthesizing further comprises synthesizing a nucleic acid that includes a normal human base sequence and synthesizing a corresponding copy of the nucleic acid that includes at least one mutation of the normal human base sequence.

15. The method as recited in claim 14, further comprising using an amplified mixture of both the nucleic acid that includes a normal human base sequence and the corresponding copy that includes at least one mutation to more accurately simulate a heterozygous state.

16. The method as recited in claim 1, further comprising adding normal human nucleic acid to the one or more synthesized nucleic acid sequences relevant for clinical reference in order to achieve a mixture of the nucleic acids representing at least a segment of homologous heterozygous alleles.

17. A method, comprising:

synthesizing a mixture of nucleic acids, wherein each nucleic acid is capable of being used as a reference in mutation testing and wherein a first end of each nucleic acid in the mixture includes a base sequence complementary to a base sequence of a first primer and a second end of each nucleic acid in the mixture includes a base sequence matching a base sequence of a second primer; and

amplifying the mixture of nucleic acids during at least one polymerase chain reaction using the first primer and the second primer.

18. The method as recited in claim 17, wherein at least one of the nucleic acids is capable of being used as a reference by including at least one mutation of a normal base sequence.

19. The method as recited in claim 17, further comprising amplifying the mixture of nucleic acids through multiple polymerase chain reaction cycles to produce an ongoing supply of the mixture.

20. The method as recited in claim 17, further comprising using the mixture of nucleic acids as at least one reference in a mutation test.

21. The method as recited in claim 17, further comprising joining multiple nucleic acid segments using a ligation extension to make at least one of the reference nucleic acids in the mixture.

22. The method as recited in claim 21, wherein for at least one of the reference nucleic acids, the synthesizing includes:

synthesizing a first nucleic acid that includes a first end comprising a base sequence complementary to the base sequence of the first primer and a second end complementary to a base sequence on a first end of a bridge nucleic acid;

synthesizing a second nucleic acid that includes a first end comprising a base sequence that matches the base sequence of the second primer and a second end complementary to a second end of the bridge nucleic acid; and

making the reference nucleic acid by joining multiple nucleic acid segments in the ligation extension, including joining the first nucleic acid on one end of the

joined segments using the bridge nucleic acid and joining the second nucleic acid on the opposite end of the joined segments using the bridge nucleic acid.

23. The method as recited in claim 17, further comprising joining multiple nucleic acids using an overlap extension to make at least one of the reference nucleic acids in the mixture.

24. The method as recited in claim 23, wherein for at least one of the reference nucleic acids, the synthesizing includes:

synthesizing a first nucleic acid that includes a first end comprising a base sequence complementary to the base sequence of the first primer;

synthesizing a second nucleic acid that includes a first end comprising a base sequence complementary to the base sequence of the second primer and a second end complementary to an end of a nucleic acid segment to be adjacent to the second nucleic acid in the final reference nucleic acid; and

making the final reference nucleic acid by joining multiple nucleic acid segments in overlap extensions, including joining the first nucleic acid on one end of the joined segments using the overlap extension and joining the second nucleic acid on the opposite end of the joined segments using the overlap extension in the absence of the first and second primers.

25. A method of propagating a reference nucleic acid, comprising:

synthesizing a single-stranded reference nucleic acid;

tagging the reference nucleic acid to make a tagged reference nucleic acid, comprising:

synthesizing a first nucleic acid tag bound to a first end of the reference nucleic acid, wherein the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set and

synthesizing a second nucleic acid tag bound to a second end of the reference nucleic acid, wherein the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set; and

subjecting the tagged reference nucleic acid to a polymerase chain reaction in the presence of the first primer and the second primer.

26. The method as recited in claim 25, wherein the first primer produces copies of a single-stranded nucleic acid complementary to the tagged reference nucleic acid and the second primer produces copies of the tagged reference nucleic acid.

27. The method as recited in claim 25, wherein the first nucleic acid tag is bound to the 3' end of the reference nucleic acid and the second nucleic acid tag is bound to the 5' end of the reference nucleic acid.

28. The method as recited in claim 25, wherein the synthesizing and the tagging include a ligation extension of two or more nucleic acids.

29. The method as recited in claim 25, wherein the synthesizing and the tagging include an overlap extension of two or more nucleic acids.

30. A method of propagating a mixture of reference nucleic acids, comprising:

synthesizing multiple single-stranded reference nucleic acids;

tagging each of the multiple reference nucleic acids to make tagged reference nucleic acids, comprising:

synthesizing a copy of a first nucleic acid tag bound to a first end of each of the multiple reference nucleic acids, wherein each copy of the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set and

synthesizing a copy of a second nucleic acid tag bound to a second end of each of the multiple reference nucleic acids, wherein each copy of the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set;

mixing the multiple tagged reference nucleic acids with each other; and

subjecting the mixture of tagged reference nucleic acids to a polymerase chain reaction in the presence of the first primer and the second primer.

31. The method as recited in claim 30, wherein the first primer produces single-stranded nucleic acids complementary to each of the multiple tagged reference nucleic acids and the second primer produces copies of each of the multiple tagged reference nucleic acids.

32. The method as recited in claim 30, wherein each copy of the first nucleic acid tag is bound to the 3' end of each of the multiple reference nucleic acids and each copy of the second nucleic acid tag is bound to the 5' end of each of the multiple reference nucleic acids.

33. The method as recited in claim 30, wherein each of multiple reference nucleic acids in the mixture of reference nucleic acids include at least a base sequence that represents a different mutation of at least part of a gene.

34. The method as recited in claim 33, wherein the mixture is used in mutation testing.

35. The method as recited in claim 34, wherein the mutation testing comprises testing a base sequence from a cystic fibrosis transmembrane conductance regulator gene.

36. A method of synthesizing a tagged reference nucleic acid for propagation, comprising:

synthesizing a single-stranded reference nucleic acid;

synthesizing a first nucleic acid tag bound to a first end of the reference nucleic acid, wherein the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set.

37. The method of synthesizing as recited in claim 36, further comprising synthesizing two of the single-stranded reference nucleic acids, wherein the two are complementary.

38. The method as recited in claim 36, wherein a single-stranded reference nucleic acid includes a mutation of a normal human nucleic acid sequence.

39. The method as recited in claim 36, further including synthesizing a second nucleic acid tag bound to a second end of the reference nucleic acid, wherein the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

40. The method as recited in claim 36, wherein the reference nucleic acid includes a base sequence representing a mutation of a cystic fibrosis transmembrane conductance regulator gene sequence for cystic fibrosis mutation testing.

41. A method of synthesizing a mixture of tagged reference nucleic acids for propagation, comprising:

synthesizing multiple single-stranded reference nucleic acids;

tagging each of the multiple reference nucleic acids to make tagged reference nucleic acids, comprising:

synthesizing a copy of a first nucleic acid tag bound to a first end of each of the multiple reference nucleic acids, wherein each copy of the first nucleic

acid tag has a base sequence complementary to a base sequence of a first primer of a primer set and

synthesizing a copy of a second nucleic acid tag bound to a second end of each of the multiple reference nucleic acids, wherein each copy of the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

42. The method as recited in claim 41, wherein each of the multiple reference nucleic acids in the mixture of reference nucleic acids represent a different mutation of a normal human nucleic acid.

43. The method as recited in claim 41, wherein each of multiple reference nucleic acids in the mixture of reference nucleic acids includes at least one base that represents a mutation of a cystic fibrosis transmembrane conductance regulator gene for cystic fibrosis mutation testing.

44. The method as recited in claim 43, wherein each of the multiple reference nucleic acids in the mixture represents a different mutation of the cystic fibrosis transmembrane conductance regulator gene.

45. The method as recited in claim 41, wherein each reference nucleic acid in the mixture includes approximately the same number of bases.

46. The method as recited in claim 41, wherein for each of the multiple reference nucleic acids, approximately the same number of bases is used between

a part of each reference nucleic acid representing a mutation and a part of each reference nucleic acid representing a nucleic acid tag.

47. The method as recited in claim 46, wherein at least one of the multiple reference nucleic acids in the mixture includes bases that represent multiple mutations of a normal human nucleic acid.

48. The method as recited in claim 47, wherein approximately the same number of bases are used between a part of a reference nucleic acid representing a first mutation and a part of the reference nucleic acid representing a second mutation as are used between each part of the reference nucleic acid representing a mutation and a part of each reference nucleic acid representing a nearest nucleic acid tag.

49. The method as recited in claim 47, wherein if the mixture includes multiple reference nucleic acids each representing multiple mutations, then the number of bases separating bases representing different mutations is approximately equal for each of the multiple reference nucleic acids representing multiple mutations.

50. The method as recited in claim 41, wherein a reference nucleic acid representing multiple mutations of a nucleic acid is ligated from at least two reference nucleic acids each representing a single mutation of the gene.

51. A tagged reference nucleic acid for a polymerase chain reaction amplification, comprising:

a synthesized reference nucleic acid having a base sequence capable of being used as a reference;

a first nucleic acid tag bound to a first end of the synthesized reference nucleic acid, wherein the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set; and

a second nucleic acid tag bound to a second end of the synthesized reference nucleic acid, wherein the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

52. The tagged reference nucleic acid as recited in claim 51, wherein the synthesized reference nucleic acid includes a base sequence representing a mutation of a gene.

53. The tagged reference nucleic acid as recited in claim 52, wherein the gene comprises a cystic fibrosis transmembrane conductance regulator gene.

54. A mixture of tagged reference nucleic acids for a polymerase chain reaction amplification, comprising:

multiple synthetic reference nucleic acids;

a copy of a first nucleic acid tag bound to a first end of each of the multiple synthetic reference nucleic acids, wherein each copy of the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set; and

a copy of a second nucleic acid tag bound to a second end of each of the multiple synthetic reference nucleic acids, wherein each copy of the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

55. A PCR amplifiable mixture of synthetic reference nucleic acids for genetic testing, comprising:

multiple synthetic reference nucleic acids each including a base sequence that represents a different site on a gene;

a copy of a first nucleic acid tag bound to a first end of each of the multiple synthetic reference nucleic acids, wherein each copy of the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer set; and

a copy of a second nucleic acid tag bound to a second end of each of the multiple synthetic reference nucleic acids, wherein each copy of the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

56. One or more pairs of base sequences for producing one or more pairs of oligomers to act as nucleic acid priming sites for propagating one or more mixtures of synthetic reference nucleic acids capable of being combined in a single mixture and amplified in a polymerase chain reaction using one or more primer sets corresponding to the one or more base sequences, comprising:

a unique first base sequence for each of the one or more pairs of base sequences to produce copies of a first type of oligomer for use as a first type of

nucleic acid priming site, wherein an end of each of the first type of oligomer is bound to a first end of each of the reference nucleic acids in the mixture of reference nucleic acids and wherein the first base sequence is complementary to a base sequence of a first primer of the two primers; and

a unique second base sequence for each of the one or more pairs of base sequences to produce copies of a second type of oligomer for use as a second type of nucleic acid priming site, wherein an end of each of the second type of oligomer is bound to a second end of each of the reference nucleic acids in the mixture of reference nucleic acids and wherein the second base sequence matches a base sequence of a second primer of the two primers.

57. The pair of base sequences as recited in claim 56, wherein if one or more of the synthetic reference nucleic acids in the mixture represent one or more mutations, then each base sequence of each member of the pair is distinct from base sequences of the synthetic reference nucleic acids in the mixture and distinct from each base sequence representing a mutation.

58. The pair of base sequences as recited in claim 56, wherein each of the base sequences in the pair are derived from a non-human source for design of tags to be bonded to a synthetic reference nucleic acid associated with a human gene.

59. A primer set for amplifying a mixture of synthetic reference nucleic acids in a polymerase chain reaction, comprising:

a first primer having a base sequence complementary to a nucleic acid priming site bound to a first end of each of the synthetic reference nucleic acids in the mixture of reference nucleic acids; and

a second primer having a base sequence matching a nucleic acid priming site bound to a second end of each of the synthetic reference nucleic acids in the mixture of reference nucleic acids.

60. A kit including one or more primer pair designs and corresponding tag designs for manufacturing multiple mixtures of synthetic reference nucleic acids within a single mixture capable of being amplified by a polymerase chain reaction, comprising:

for each of the multiple mixtures: first and second tag designs for nucleic acid oligomers each having a base sequence unique over base sequences included in the synthetic reference nucleic acids in the mixture;

a first primer design:

wherein the first primer has a base sequence complementary to the base sequence of the first tag,

wherein copies of the first tag are bondable to a first end of each synthetic reference nucleic acid in the mixture, and

wherein each copy of the first tag comprises a nucleic acid priming site for the first primer; and

a second primer design:

wherein the second primer has a base sequence matching the base sequence of the second tag,

wherein copies of the second tag are bondable to a second end of each synthetic reference nucleic acid in the mixture, and

wherein each copy of the second tag provides a template for the first primer to produce a nucleic acid priming site for the second primer.

61. A method, comprising:

synthesizing multiple reference nucleic acids, wherein each of the multiple reference nucleic acids includes at least one mutation in a base sequence of a cystic fibrosis transmembrane conductance regulator (CFTR) gene;

synthesizing a copy of a first nucleic acid tag bound to a first end of each of the multiple reference nucleic acids, wherein each copy of the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer pair;

synthesizing a copy of a second nucleic acid tag bound to a second end of each of the multiple reference nucleic acids, wherein each copy of the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

62. The method as recited in claim 61, further comprising inserting approximately a consistent number of bases between each base representing a mutation on one of the reference nucleic acids and one of the tags on the reference nucleic acid.

63. The method as recited in claim 62, further comprising subjecting the mixture of tagged reference nucleic acids to a polymerase chain reaction in the presence of the first primer and the second primer.

64. A mixture of reference nucleic acids, comprising:

multiple reference nucleic acids, wherein each of the multiple reference nucleic acids is capable of being used as a clinical reference;

a copy of a first nucleic acid tag bound to a first end of each of the multiple reference nucleic acids, wherein each copy of the first nucleic acid tag has a base sequence complementary to a base sequence of a first primer of a primer pair; and

a copy of a second nucleic acid tag bound to a second end of each of the multiple reference nucleic acids, wherein each copy of the second nucleic acid tag has a base sequence matching a base sequence of a second primer of the primer set.

65. The mixture of reference nucleic acids as recited in claim 64, further comprising total genomic DNA from a reference human source that is known to differ at at least one locus from one or more of the multiple reference nucleic acids.

66. The mixture of reference nucleic acids as recited in claim 65, wherein the total genomic DNA from a reference human source includes no known mutations.

67. A system for genetic mutation testing, comprising:

a means for inputting a patient genetic sample;

a reference nucleic acid mixture;

a means for comparing the patient genetic sample against at least one constituent of the reference nucleic acid mixture; and

a means for displaying the comparison.

68. The system for genetic mutation testing as recited in claim 67, wherein the reference nucleic acid mixture is used to calibrate at least one part of the system.

69. The system for genetic mutation testing as recited in claim 67, wherein the reference nucleic acid mixture is used to verify at least one part of the system.

70. The system for genetic mutation testing as recited in claim 67, wherein the reference nucleic acid mixture is used to verify another reagent used in the system.

71. A method, comprising:

synthesizing a first mixture of various reference nucleic acids, wherein each of the various reference nucleic acids in the first mixture includes one or more tags allowing PCR amplification of the first mixture via a primer set specific to the tags of the first mixture; and

synthesizing a second mixture of various reference nucleic acids, wherein each of the various reference nucleic acids in the second mixture includes one or more tags allowing PCR amplification of the second mixture via a second primer set specific to the tags of the second mixture.

72. The method as recited in claim 71, further comprising combining the first and second mixtures to make a single mixture and differentially amplifying the first mixture and the second mixture in a PCR reaction by controlling amounts of the first primer set and the second primer set in the single mixture.

73. The method as recited in claim 72, wherein at least some of the reference nucleic acids include mutations of a normal human nucleic acid.

74. The method as recited in claim 73, further comprising adding normal human nucleic acid to the single mixture to obtain heterozygous pairs, wherein each heterozygous pair includes a normal segment of human nucleic acid and a mutated copy of the normal segment of human nucleic acid.